SI Appendix for:

A MYC2/MYC3/MYC4-dependent transcription factor network regulates water spray-responsive gene expression and jasmonate levels

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Supplementary Datasets.

Dataset S1. Processed RNAseq data for *myc234* 25 min after water spray.

Dataset S2. Differentially expressed genes in Col-0 after 25 min.

Dataset S3. Differentially expressed genes (>100 fold) in Col-0 after 25 min.

Dataset S4. RNA-seq expression levels of the 9 selected water spray-induced transcription factors affected by *myc234*

Dataset S5. Processed RNAseq data for *myc2 pMYC2:MYC2-FLAG* time course after water spray.

Dataset S6. Differentially expressed genes in *myc2 pMYC2:MYC2-FLAG*.

Dataset S7. Core touch-induced transcripts.

Dataset S8. *myc2 pMYC2:MYC2-FLAG* ChIP-seq

Dataset S9. Core MYC2-dependent regulon.

Dataset S10. Presence of G-boxes in MYC2-regulated promoters

Dataset S11. Differentially expressed proteins in *myc2 pMYC2:MYC2-FLAG*.

Dataset S12. Water spray-induced expression of hormone biosynthesis genes.

Dataset S13. Hormone quantification after water spray treatment

Dataset S14. DNA oligos used in this study.

Dataset S15. Internal standards used for hormone quantification.

Dataset S16. Processed proteomic spectral counting data for *myc2 pMYC2:MYC2-FLAG* time course after water spray.

Supplementary video files

Video S1. An example of water spray treatment performed in this study, using distilled water and a household spray bottle.

Video S2. An example of touch by gentle brush treatment performed in this study, using a soft paint brush.

Supplementary Figures

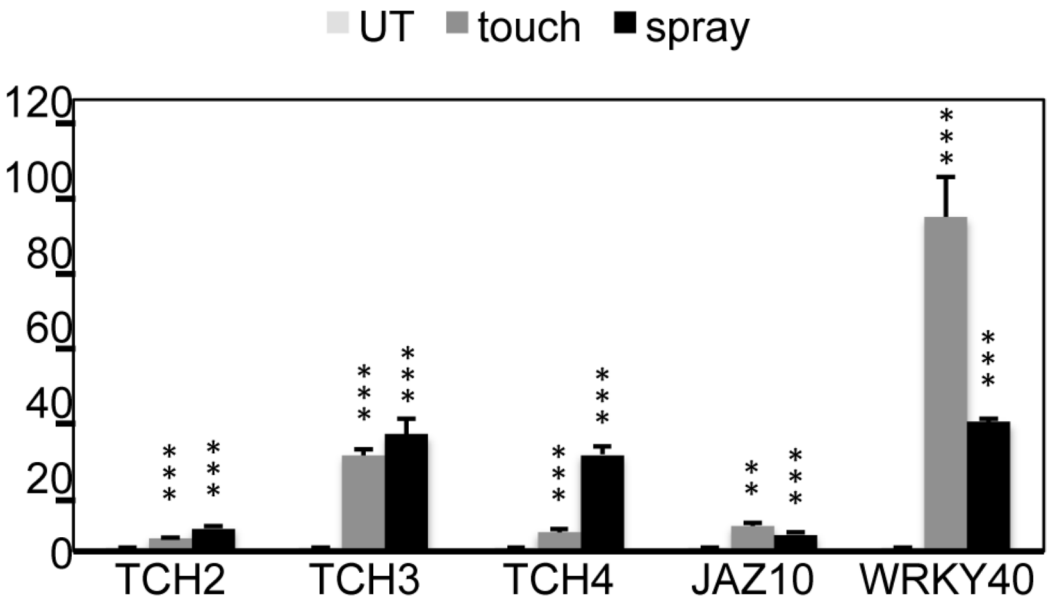

Fig. S1. Transcript accumulation levels of selected touch-responsive genes 25 min after touch by blunt tweezers or spray compared to untouched (UT) Col-0 seedlings. The Y-axis represents normalised fold-induction (UT set to 1). The error bars designate SE of the mean (n = 3). Statistical significance was determined independently for the touch and spray treatment compared to UT by the Student's t-test (**P < 0.005, ***P < 0.0005).

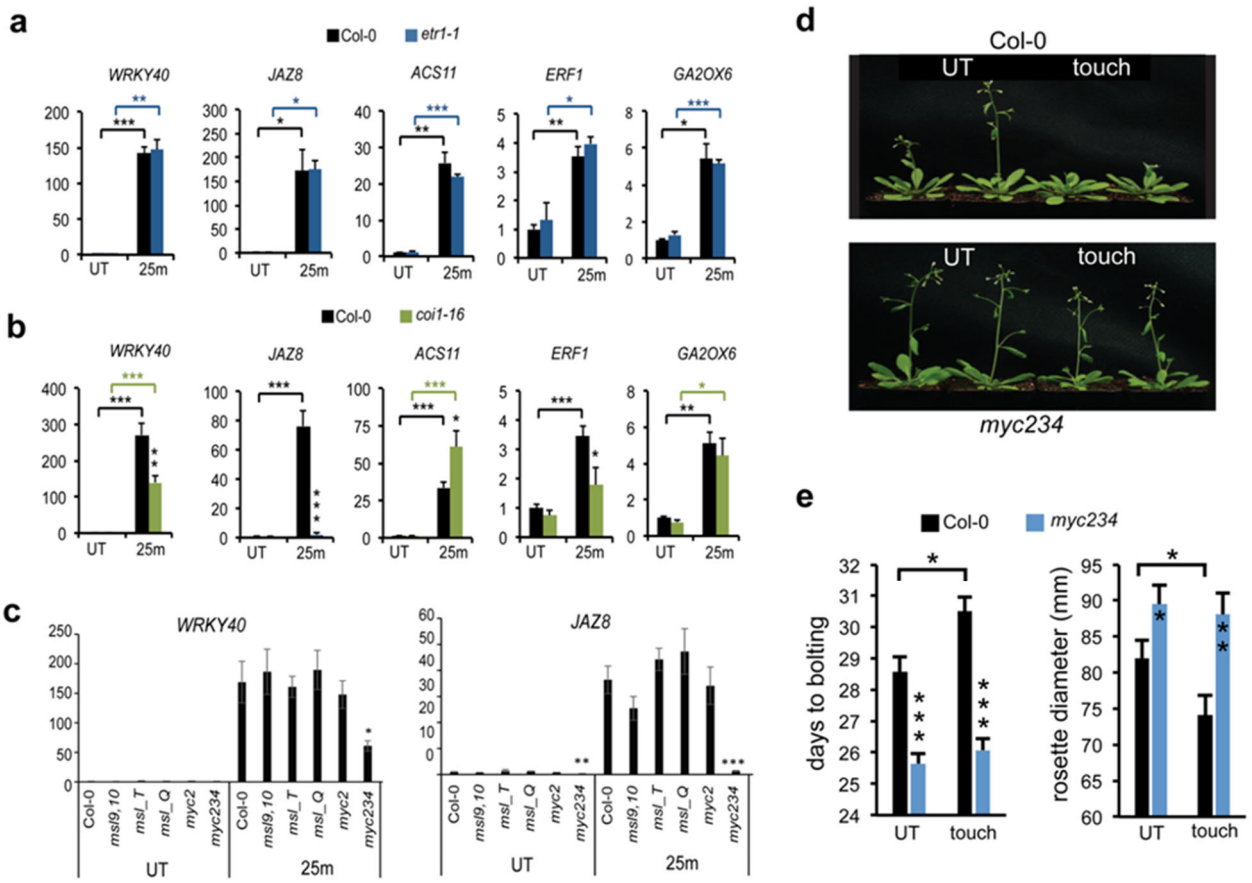

Fig. S2. *myc234* mutants show reduced spray-responsive gene expression and do not display a touch-induced phenotype. (*A, B*) Transcript analysis by qPCR of selected touch genes in seedlings of Col-0 (black bars) and the *etr1-1* mutant (A, blue bars) or *coi1-16* (*B*, green bars) untouched (UT) or after 25 min (25m) spray treatment. Values on the Y-axis represent fold-changes compared to UT Col-0 (set to 1). The error bars designate SE of the mean (n = 4). Statistical significance was determined using Student's *t*-test between genotypes and treatments (**P* < 0.05, ***P* < 0.005, ****P* < 0.0005). (*C*) Transcript analysis by QPCR of *WRKY40* and *JAZ8* in seedlings of Col-0, *msl9msl10*, *msl4msl5msl6* (*msl_T*), *msl4msl5msl6msl9msl10* (*msl_Q*), *myc2* and *myc234* untouched (UT) or after 25 min (25m) spray treatment. The error bars designate SE of the mean (n = 3-4). Statistical significance was determined using Student's t-test between mutants and Col-0 within a treatment (**P* < 0.05, ***P* < 0.005, ****P* < 0.0005). (*D*) 14-day-old untouched and touched by tweezers Col-0 and *myc234* plants. (*E*) Left: average days to bolting (left panel) and rosette diameter at day 33 (right panel) in Col-0 and *myc234* untouched (UT) or daily touched twice for 1 min. The error bars designate SE of the mean (n = 16). Statistical significance was determined using Student's t-test (**P* < 0.05, ***P* < 0.005, ***P < 0.0005).

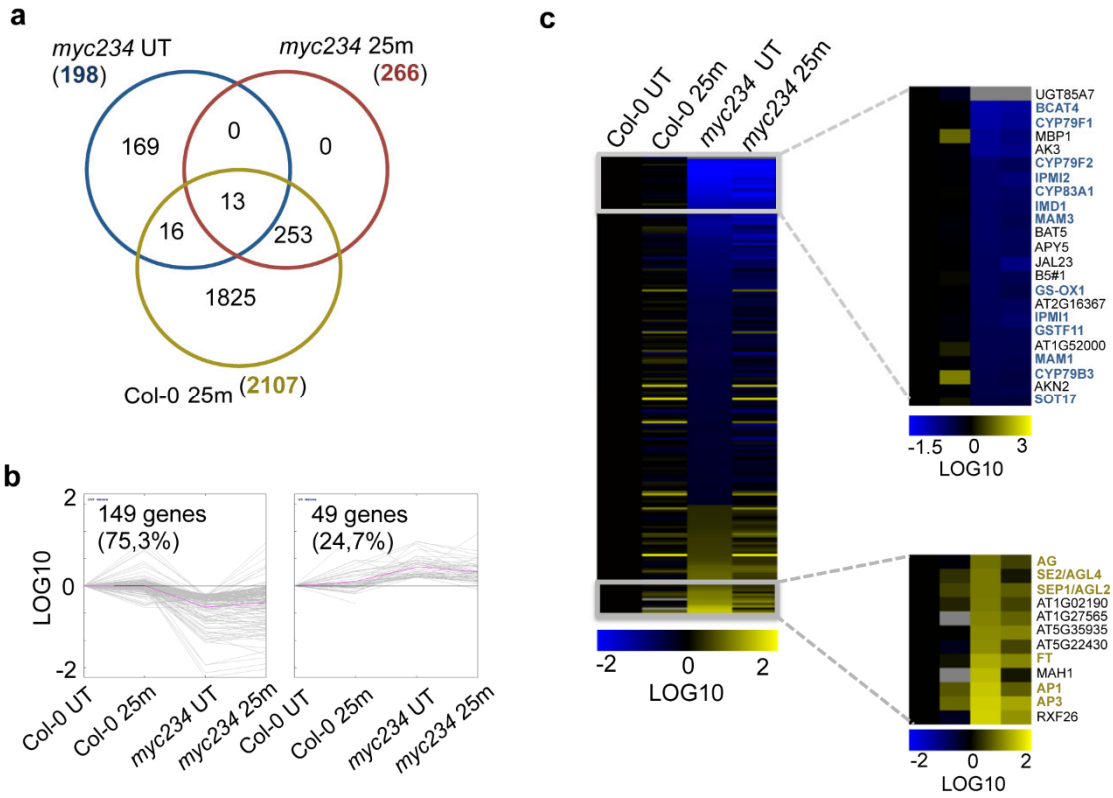

Fig. S3. Transcript profiling of genes differentially expressed in untreated *myc234* mutants compared to untreated Col-0. (*A*) Venn diagram showing the connection between genes differentially expressed in untreated *myc234* seedlings (*myc234* UT; blue), genes differentially expressed in water-sprayed *myc234* seedlings (*myc234* 25m; red) and genes differentially expressed in water-sprayed Col-0 seedlings (Col-0 25m, yellow). (*B*) *k*-means clustering of the 198 MYC2/MYC3/MYC4-dependent genes in untouched seedlings showing the numbers and dynamics of down-regulated and up-regulated genes. The x-axis shows the different genotypes untouched (UT) and sampled 25 min after spray (25m). The Y-axis represents LOG10-transformed fold-induction. (*C*) Heat-map of the subset of 198 genes differentially expressed (> 2-fold up or down; $P < 0.05$) in untreated (UT) in *myc234* seedlings compared to Col-0 (set to 1), sorted from lowest to highest expression (*myc234* UT). Values are LOG10-transformed and blue and yellow denote down-regulation and up-regulation, respectively. Insets show the most-highly down-regulated (> 7.5-fold) and up-regulated (> 20-fold) genes. The genes indicated in blue and yellow are glucosinolate genes and flower development and flowering time genes, respectively.

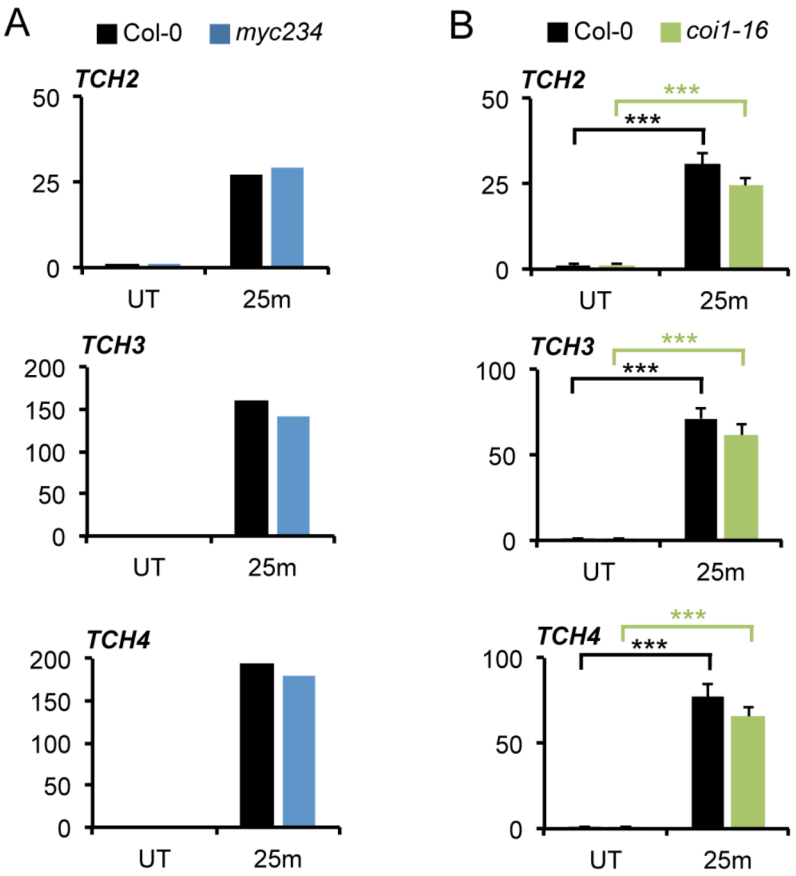

Fig. S4. Transcript accumulation levels of *TCH* genes in *coi1-16* and *myc234*. (*A*) RNA-Seq analysis on seedlings of seedling of Col-0 and *myc234* untouched (UT) and 25 min after water spray (25m). The Y-axis represents normalised fold-induction (UT Col-0 set to 1). Differences between treatments and genotypes are significant and non-significant, respectively (see Methods and Dataset S1). (*B*) qPCR analysis on seedling of Col-0 and *coi1-16* untouched (UT) and 25 min after water spray (25m). The Y-axis represents normalised fold-induction (UT Col-0 set to 1). The error bars are SEs of the mean ($n = 3$ for UT and $n = 4$ for 25m). Statistical significance between treatments and genotypes was determined by the Student's t-test (**$P < 0.005$, ***$P < 0.0005$).

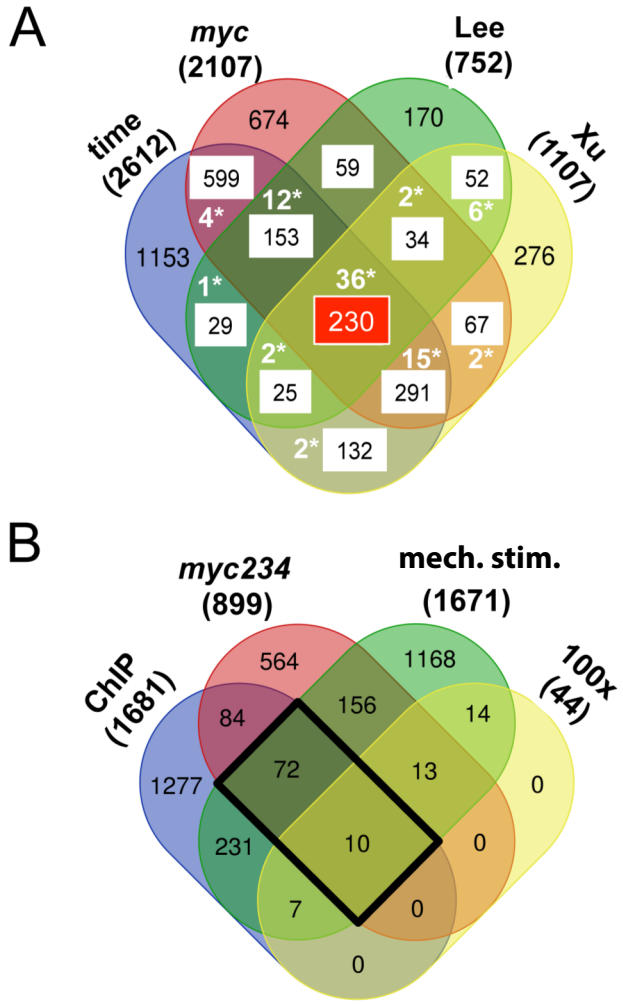

Fig. S5. The defined core mechanical stimulation transcriptome and MYC2 regulon. (*A*) Venn-diagram showing the relationship between the RNA-Seq analyses in Fig. 1 (*myc*), Fig. 3 (time), Lee (14) and Xu (15). Transcript numbers present in at least 2 out of 4 analyses are boxed in white and represent our defined core touch transcriptome. Boxed in red are mechanical stimulation-responsive transcripts present in all four data sets. The remaining transcripts are specific to a single RNA-Seq experiment. Marked in white with asterisks are genes present in the MYC2 regulon indicated in panel b. Number of genes in each group is indicated between brackets. (*B*) Venn-diagram showing the relationship between the genes bound by MYC2 25m after water spray in the ChIP-Seq (ChIP), significantly affected by *myc234* after water spray relative to Col-0 ($P < 0.05$; myc), the defined 'core mechanical stimulation transcriptome' in panel a (mech. stim.) and the genes over 100-fold induced 25min after spray in Col-0 (100x). Boxed is the defined MYC2 regulon. Number of genes in each group is indicated between brackets.

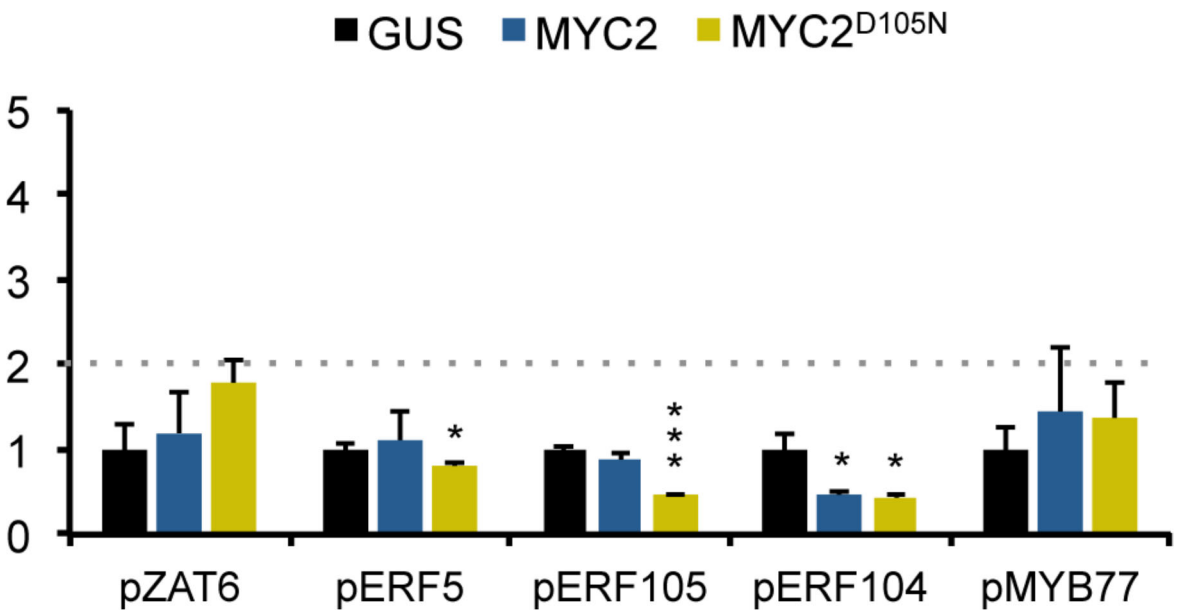

Fig. S6. *Trans*-activation of touch TF promoters by MYC2. Agrobacterium-mediated trans-activation assays in *N. tabacum* protoplast of the selected TF gene promoters by β-glucuronidase (GUS), MYC2 and MYC2^{D105N}. The Y-axes represents normalised firefly luciferase activity (fLUC/renilla luciferase). Values represent fold-induction relative to the GUS control (set to 1) and are presented on top of the bars. Error bars represent SE of the mean ($n = 4$). Statistical significance was determined by Student's t test (*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$).

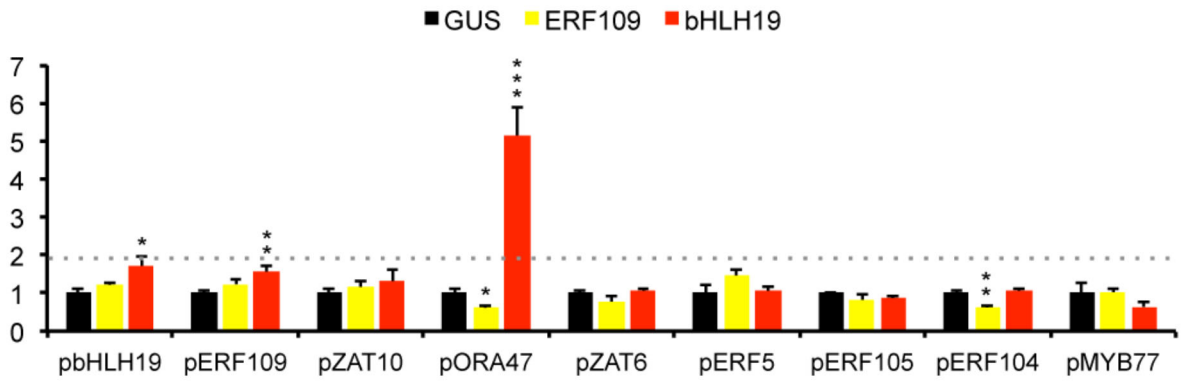

Fig. S7. *Trans*-activation of touch TF promoters by ERF109 and bHLH19. Agrobacterium-mediated trans-activation assays in *N. tabacum* protoplast of the selected TF gene promoters by β-glucuronidase (GUS), ERF109 and bHLH19. The Y-axes represents normalised firefly luciferase activity (fLUC/renilla luciferase). Values are fold-induction relative to the GUS control (set to 1) and are presented on top of the bars. Error bars represent SE of the mean (*n = 8*). Statistical significance was determined by Student's t test (**P* < 0.05, ***P* < 0.005, ****P* < 0.0005).

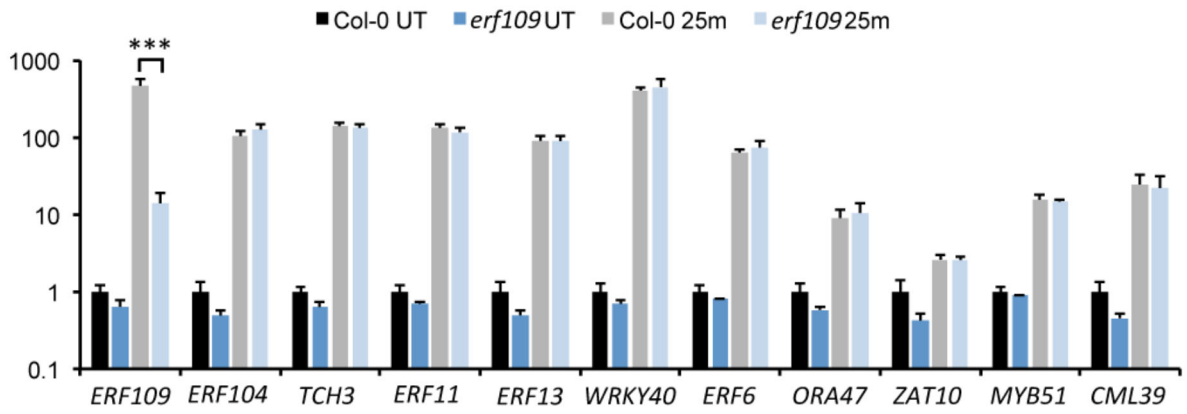

Fig. S8. Transcript accumulation levels of selected genes in *erf109.* qPCR analysis on seedling of Col-0 and *erf109* untouched (UT) and 25 min after water spray (25m). The Y-axis represents normalised fold-induction (UT set to 1). The error bars designate SE of the mean (n = 3). Statistical significance between treatments and genotypes was determined by the Student's t-test (**P < 0.005, ***P < 0.0005).

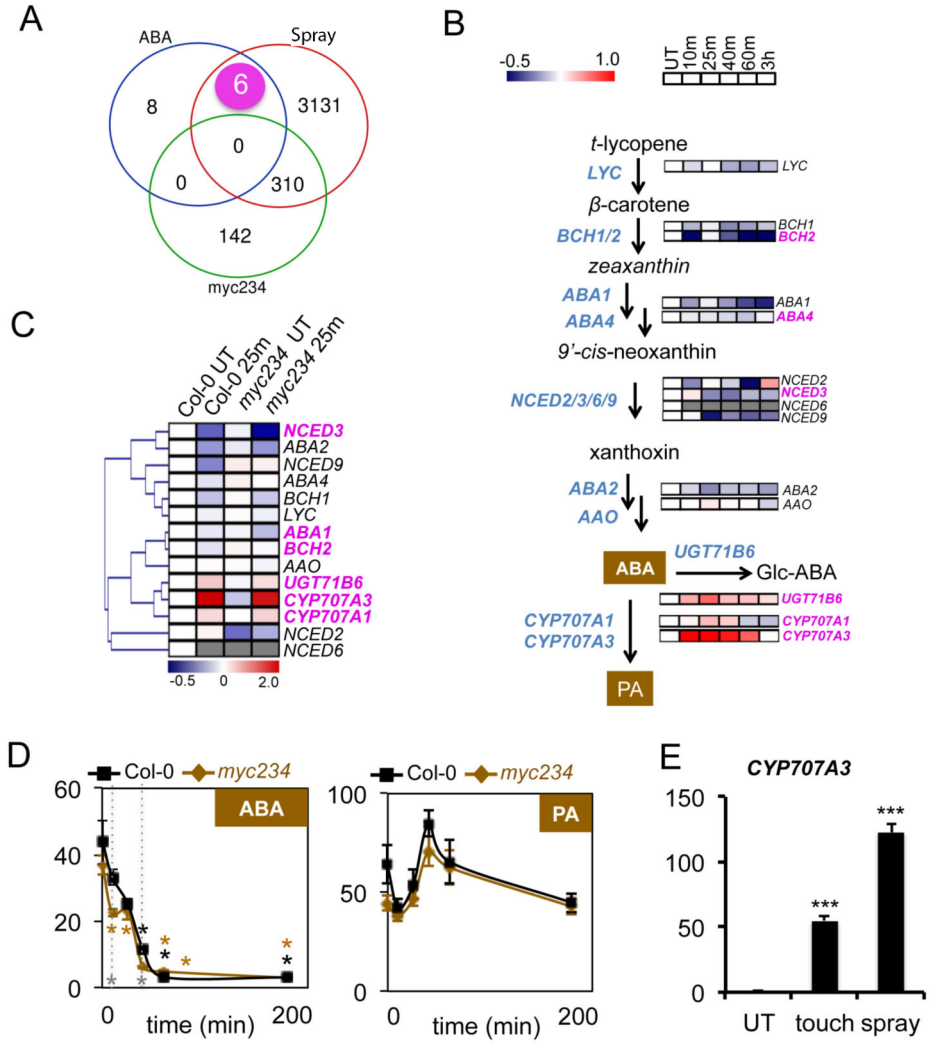

Fig. S9. ABA-related metabolite and transcript profiling of the MYC2/MYC3/MYC4-dependent water spray response. (*A*) Venn diagram showing the overlap between the ABA biosynthesis genes (ABA), MYC2/MYC3/MYC4-dependent genes (*myc234*) and water spray-responsive genes (touch). The colour code corresponds to the genes in (*B*, *C*): in pink are water spray-responsive MYC2/MYC3/MYC4-independent ABA biosynthesis genes. (*B*) Pathway for ABA biosynthesis and expression profiling of the genes encoding the depicted enzymatic steps. Measured metabolites are boxed in brown and the enzymatic steps are indicated. Visualisation of the time-course expression analysis of the ABA-biosynthesis genes are depicted right-hand side of each enzymatic step. The time-points are indicated on top and the scale bar represents LOG10 transformed values. Red and blue denote up-regulation and down-regulation, respectively. (*C*) Hierarchical cluster analysis of the ABA biosynthesis genes. Clustering was performed using transcript data shown in B as well as transcript data from Col-0 and *myc234* seedlings untouched (UT) and 25 min after touch (25m). The scale bar represents LOG10 transformed values. Red and blue denote up-regulation and down-regulation, respectively. The colour code of the transcripts are derived from the Venn diagram in (*D*). (*D*) Accumulation of ABA and PA after water spray in Col-0 and *myc234* seedlings. The Y-axis denotes pmol.g^{-1} FW. The X-axis represents sampling time (min) 10, 25, 40, 60, and 180 min after touch. Black and coloured asterisks indicate differences (Student's t-test; * $P < 0.05$; ** $P < 0.005$, *** $P < 0.0005$) with UT in Col-0 and *myc234*, respectively. Grey asterisks indicate differences between Col-0 and *myc234* at each time-point (Student' *t*-test, $P < 0.05$, ** $P < 0.005$). (*E*) Quantitative RT-PCR showing the effect of touch and spray on transcript accumulation of *CYP707A3*. Y-scale represents normalised fold-induction compared to untouched (UT; set to 1). Error bars are standard error of the mean ($n = 3$). Statistical significance was determined by the Student's t-test (***$P < 0.0005$). Abbreviations: ABA, Abscisic acid; PA, Phaseic Acid. Gene names can be found as Dataset S13.

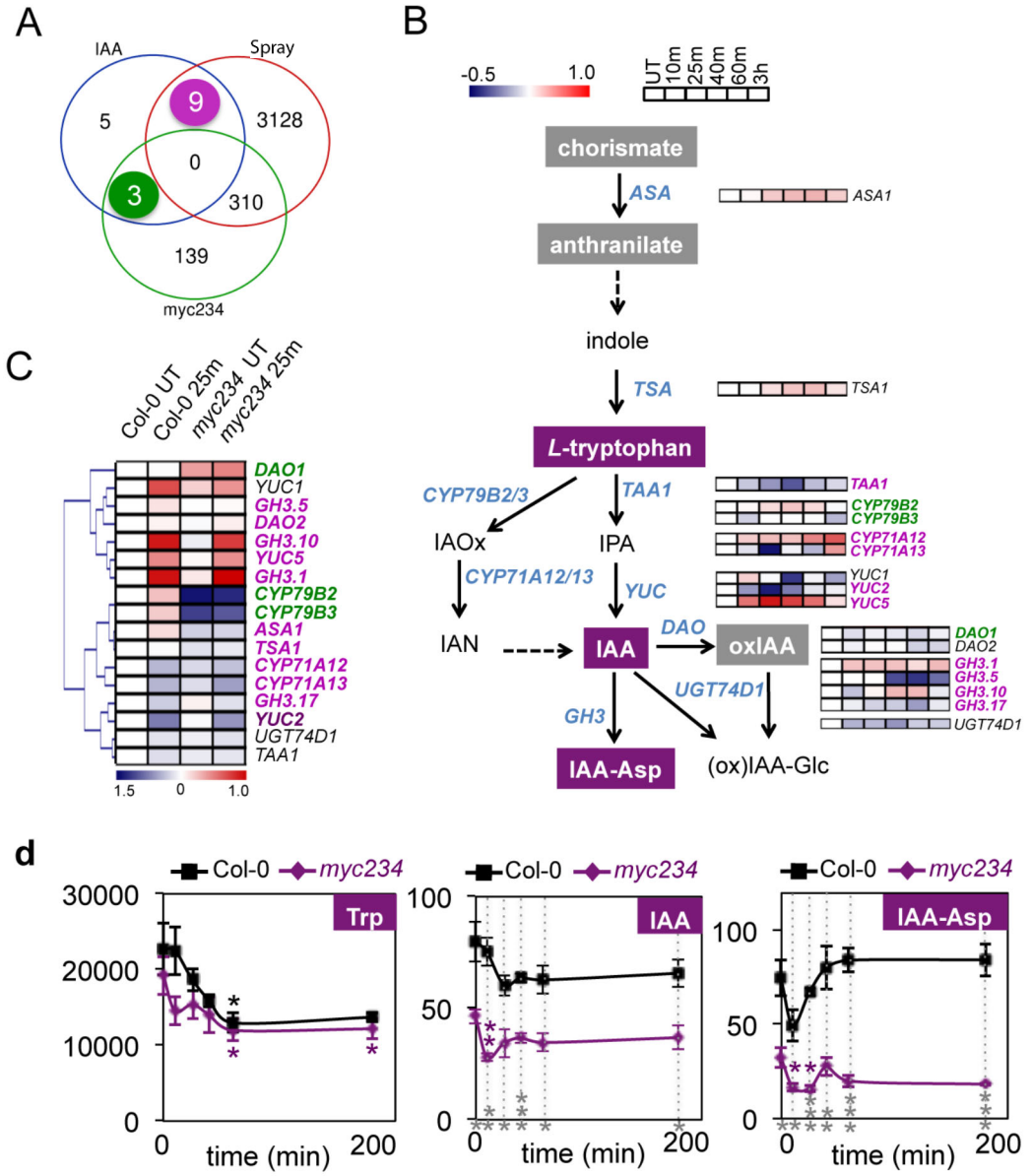

Fig. S10. IAA-related metabolite and transcript profiling of the MYC2/MYC3/MYC4-dependent water spray response. (*A*) Venn diagram with selected auxin biosynthesis genes (IAA), MYC2/MYC3/MYC4-dependent genes (*myc234*) and water spray-responsive genes (touch). The colour code corresponds to the genes in (*B*, *C*): in green are MYC2/MYC3/MYC4-dependent IAA biosynthesis genes, in blue are spray-responsive MYC2/MYC3/MYC4-dependent JA biosynthesis genes and in pink are spray-responsive MYC2/MYC3/MYC4-independent IAA biosynthesis genes. (*B*) Pathway for IAA biosynthesis and expression profiling of the genes encoding the depicted enzymatic steps. Measured metabolites are boxed in purple and the enzymatic steps are indicated. Visualisation of the time-course expression analysis of the IAA-biosynthesis genes are depicted right-hand side of each enzymatic step. The time-points are indicated on top and the scale bar represents LOG10 transformed values. Red and blue denote up-regulation and down-regulation, respectively. (*C*) Hierarchical cluster analysis of the IAA biosynthesis genes. Clustering was performed using transcript data of the time series shown in B as well as transcript data from Col-0 and *myc234* seedlings untouched (UT) and 25 min after water spray (25m). The scale bar represents LOG10 transformed values. Red and blue denote up-regulation and down-regulation, respectively. The colour code of the transcripts are derived from the Venn diagram in (*A*). (*D*) Accumulation of Trp, IAA and IAA-Asp after water spray in Col-0 and *myc234* seedlings. The Y-axis denotes pmol.g^{-1} FW. The X-axis represents sampling time (min) 10, 25, 40, 60, and 180 min after touch. Black and coloured asterisks indicate differences (Student's t-test; * $P < 0.05$; ** $P < 0.005$, *** $P < 0.0005$) with UT in Col-0 and *myc234*, respectively. Grey asterisks indicate differences between Col-0 and *myc234* at each time-point (Student's *t*-test, $P < 0.05$, ** $P < 0.005$). Abbreviations: IAA, Indole-Acetic Acid. Gene names can be found as Dataset S13.